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(51) INT CL<sup>6</sup>

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(52) UK CL (Edition N )

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**BIOSIS Acc.No. 96081026 Martindale, The Extra  
Pharmacopoeia, 29th Edn.(1988) page 1272 "Uses"**

(58) Field of Search

**UK CL (Edition N ) A5B BHA BJA**

**INT CL<sup>6</sup> A61K 31/525**

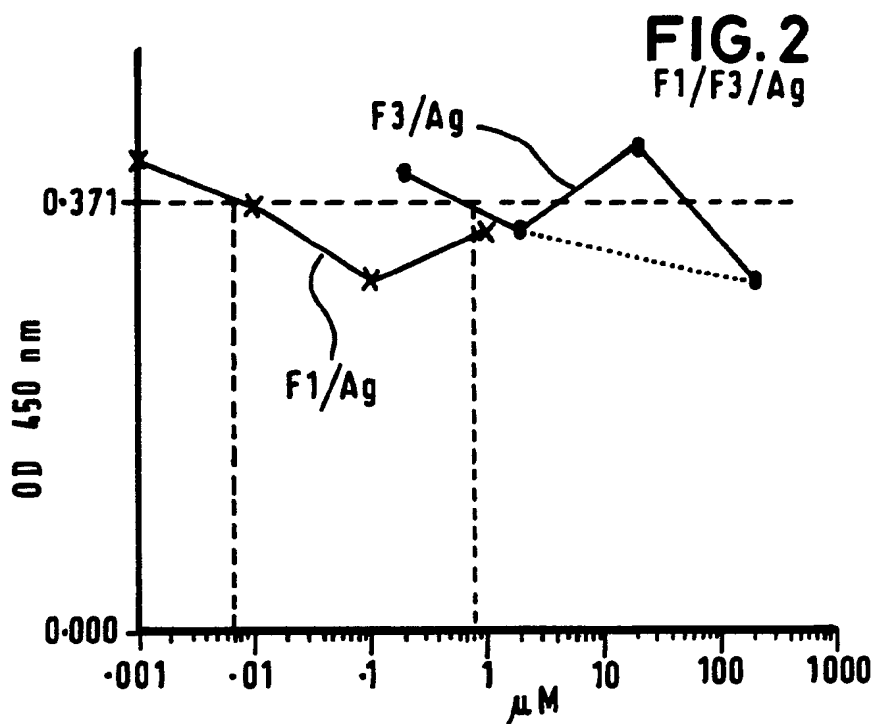
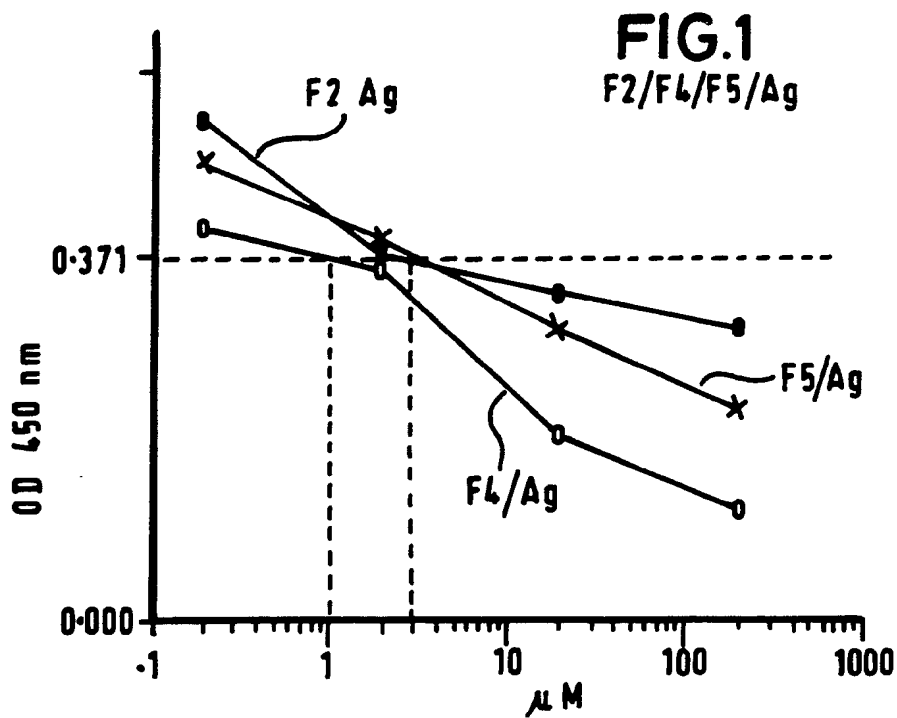
**ONLINE DATABASES: WPI, DIALINDEX (MEDICINE),  
CAS ONLINE**

(54) **Anti-viral agents comprising flavins**

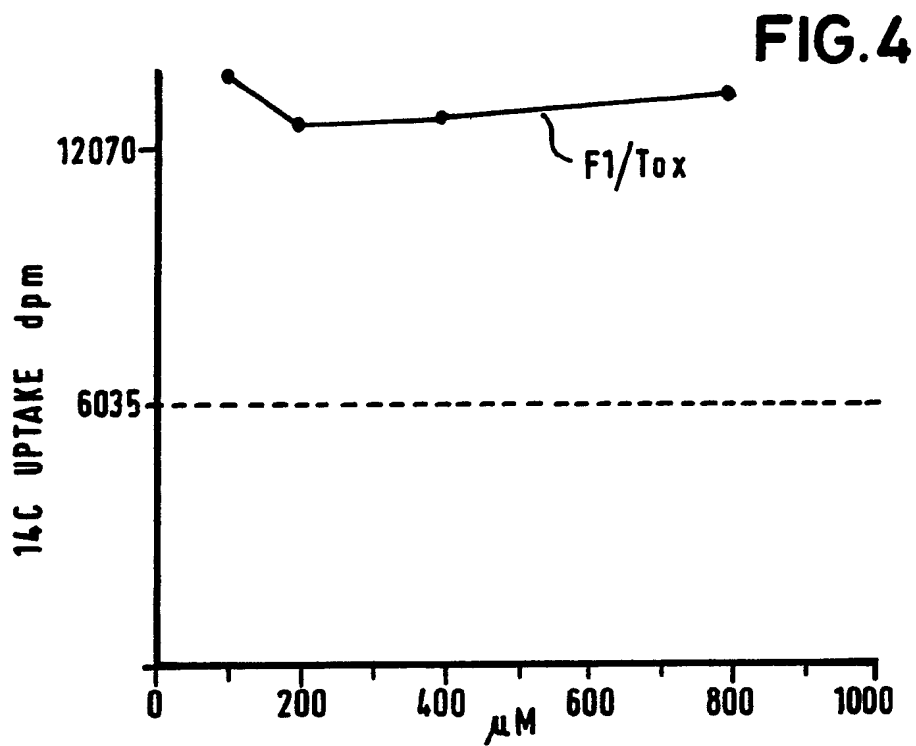
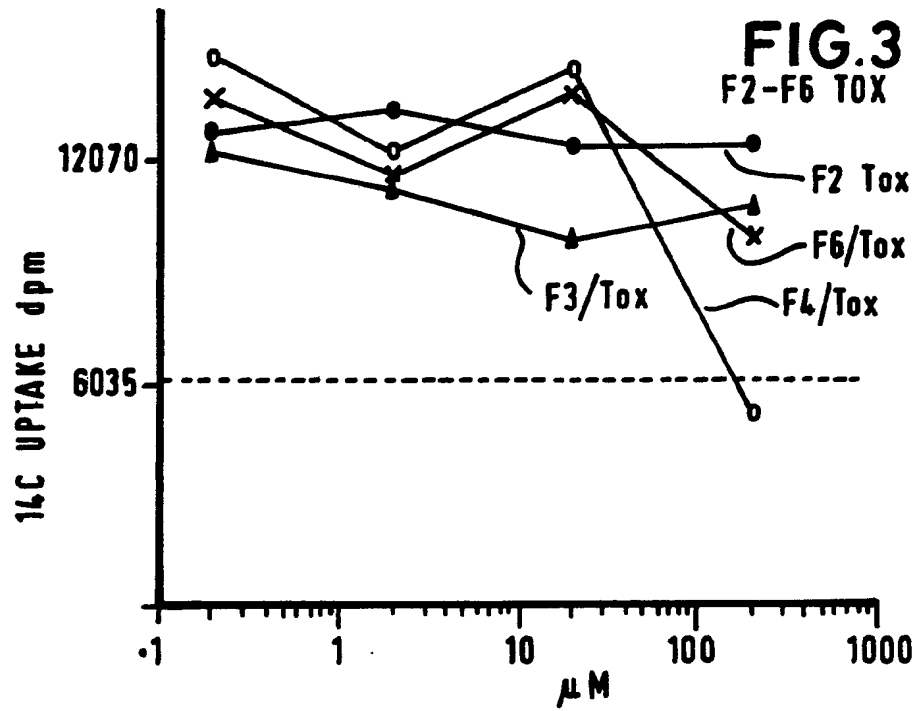
(57) Flavins and their derivatives are useful for administration to mammalian subjects as an anti-viral agents, Riboflavin and riboflavin derivatives are particularly preferred for use in the treatment of HIV infection.

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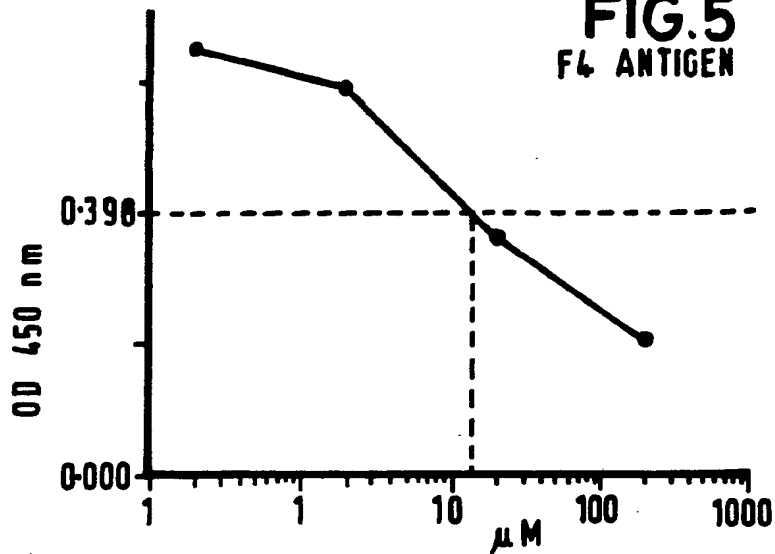


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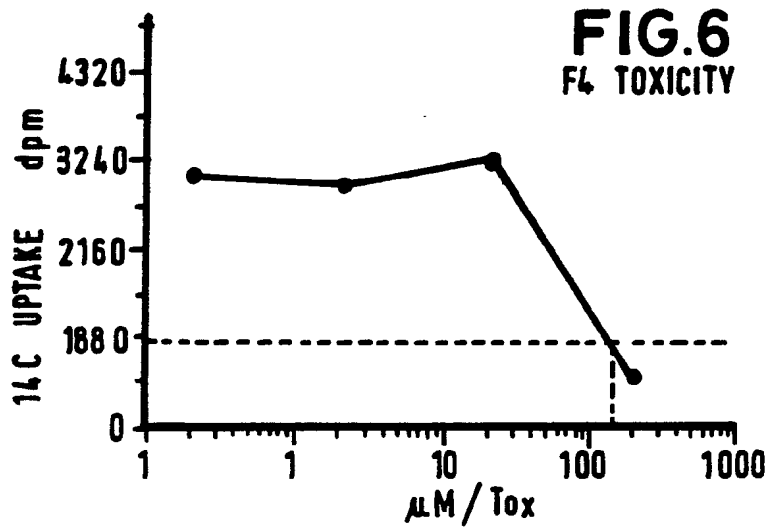


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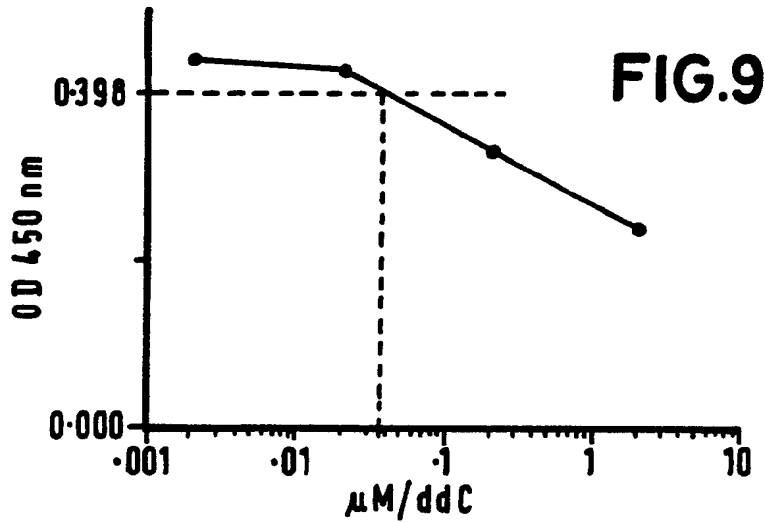
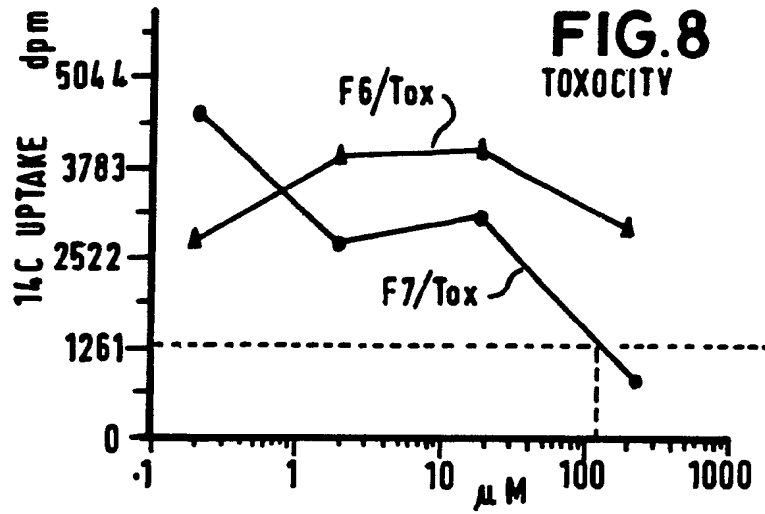
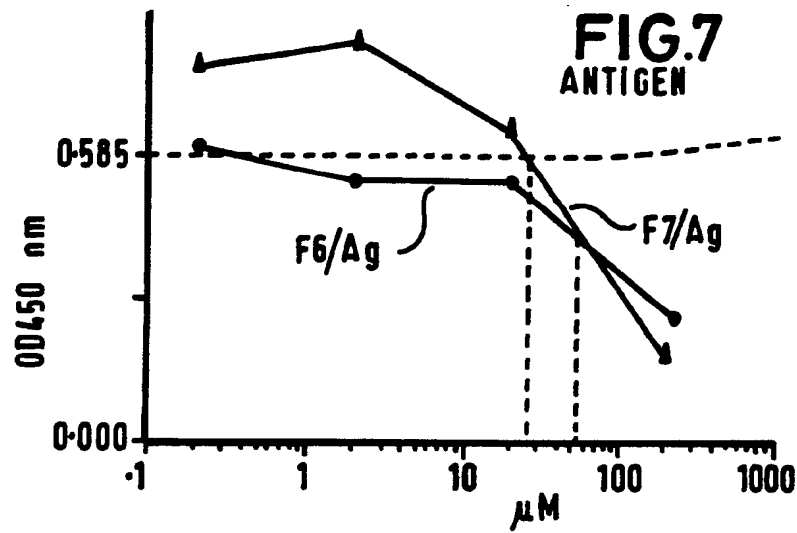
**FIG.5**  
F4 ANTIGEN



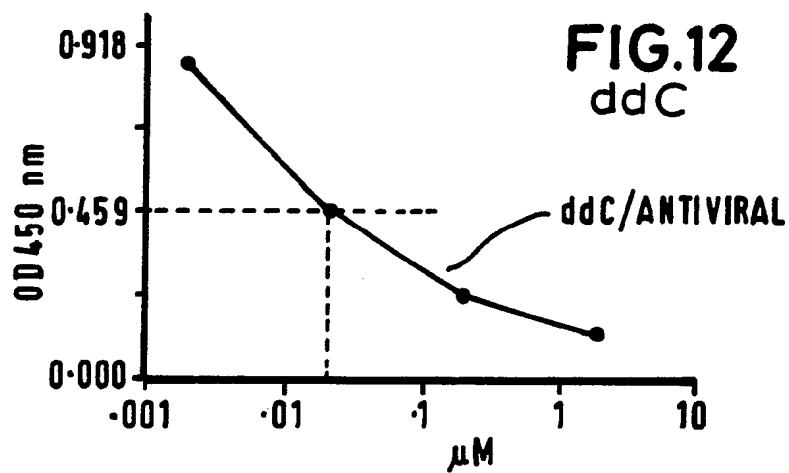
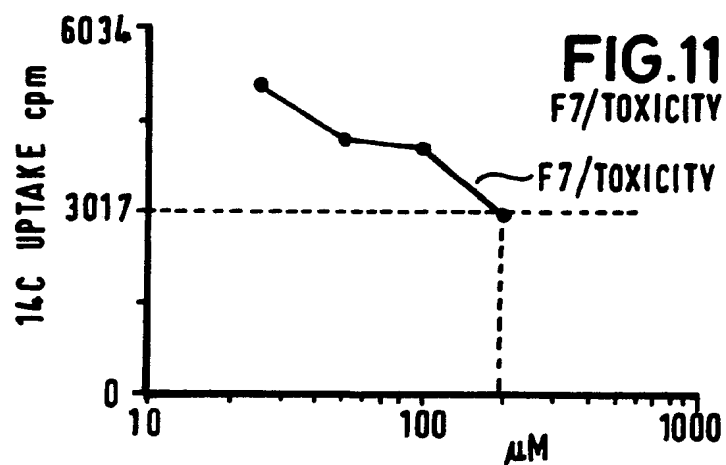
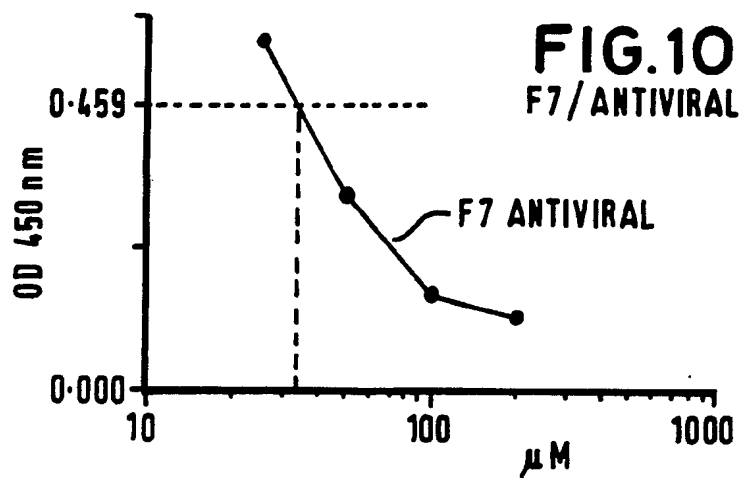
**FIG.6**  
F4 TOXICITY



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ANTI-VIRAL AGENTS

The present invention relates to anti-viral agents and their use in the treatment of human and animal patients to alleviate or cure the ill-effects caused by viral infection, especially HIV. A detailed study of compounds according to the invention has been carried out to evaluate their efficacy against infection from several strains of HIV-1. The compounds have similar activity against HIV in both acutely and chronically infected cells. This is a dual property only occasionally associated with other compounds which are in current use in the therapy of HIV infection although *de nova* (acute) infections of cells may be treated by compounds which act early in the replication cycle of HIV to block integration of vDNA into the host chromosome. It is this integration which signifies entry of the infection into the chronic state. Compounds which act post-integration of HIV are therefore inhibitors of chronically infected cells. Zidovudine (AZT) for example is only active against *de nova* infection of HIV and has no significant activity against chronically infected cells. Inhibitors of gene expression of HIV (which is a positive strand RNA virus) would therefore be active in HIV chronically infected cells.

25

HIV is a positive strand RNA virus which affects humans. The virus attaches to cell membranes by virion adsorption to CD4 surface receptor. The virion then passes through

the cell membrane penetratively and enters the cell cytoplasm. Uncoating of the virion then takes place in the cytoplasm whereby the viral envelope and the protein coat of the genome release the viral RNA into the cytoplasm.

5 Reverse transcription therein produces a double-stranded DNA transcript from host cell genetic material. This invades the host cell nucleus and integrates with the host cell chromosomal DNA. Transcription follows to produce a vRNA replicate which is translated in the cytoplasm to  
10 produce new viral proteins. The latter then assembles with vRNA at the inner cell surface to produce new virus particles which are released from the host cell.

HIV is normally associated with an initial asymptomatic  
15 phase. This initial asymptomatic phase may last a number of years before the early signs of HIV disease occur.

A number of ideas causing cell death are proposed. Apoptosis is one of these. It is a morphologically  
20 distinctive form of programmed cell death involved in many physiological and pathological processes including cellular processes which seek to maintain appropriate intracellular oxidant-antioxidant balance. Cell death in T-cells is closely associated with this balancing process. Infection  
25 with HIV is thought gradually to disturb the balance in favour of cell death. Another critical factor in determining whether cells will grow and divide in a normal fashion is intracellular ATP concentration. Low

intracellular levels of ATP are associated with ischemic death. T-lymphocytes are especially vulnerable to depletion of intracellular ATP levels. HIV infection may disturb cellular oxidative phosphorylation which is the cellular process responsible for ATP levels in the cell. Cell death from whatever cause will eventually lead to cell depletion to a level that induces AIDS.

Much of the current work in the field of antiviral research is concerned with targeting specific viral encoded enzymes. Compounds discovered from this research, in principle, should have low toxicity on cellular processes. The long term use of compounds in clinical trials in HIV infection treatment has not given the degree of benefit initially expected, and new approaches are needed.

Riboflavine is a known compound, which is also variously known as:

E101;  
Lactoflavin;  
Riboflavin;  
Riboflavinum;  
Vitamin B<sub>2</sub>;  
Vitamin G;  
7,8-Dimethyl-10-(1'-D-ribityl) isoalloxazine; and  
3,10-Dihydro-7,8-dimethyl-10-(D-ribo-2,3,4,5-tetrahydroxypentyl) benzopteridine-2,4-dione.

Riboflavine is commercially available as such or as its sodium phosphate or tetrabutyrates salt, typically in the former instance as the dihydrate salt. It is also  
5 available in various mixtures with other vitamins, all essentially being for the treatment of, inter alia, vitamin B deficiency. In such mixtures the dose of riboflavin varies between 0.5 and 10 mg, with a maximum recommended daily dose being 30 mg.

10

No adverse effects have been reported with the use of riboflavine. However, significant doses of riboflavine result in a bright yellow discoloration of the urine which may interfere with certain laboratory tests.

15

The riboflavine requirement of humans is often related to the energy intake, but it appears to be more closely related to resting metabolic requirements. A daily dietary intake of about 1.3 to 1.8 mg of riboflavine is recommended  
20 that is to say the basic recommended intake of riboflavine is 550 µg per 4200 kJ (1000 kcal) of diet - Report of a Joint FAO/WHO Expert Group, Tech. Rep. Ser. Wld 111th Org. No. 362, 1967.

25

The estimated acceptable daily intake of riboflavine is up to 500 µg per kg body weight - see Thirteenth Report of FAO/WHO Expert Committee on Food Additives, Tech. Rep. Ser. WHO. No. 445, 1971.

Riboflavine, which is a water-soluble vitamin, is essential for the utilisation of energy from food. The active, phosphorylated forms, flavine mono-nucleotide and flavine adenine dinucleotide, are involved as co-enzymes in oxidative/reductive metabolic reactions.

Various other flavins and derivatives thereof are also known, mainly as flavouring agents.

10

It has now been found surprisingly that the administration of riboflavine, as well as other flavins and derivatives thereof, at doses far higher than previously used or recommended can be highly effective in the management and treatment of viral infections, in particular HIV. The structure of the compound indicates involvement in the process of oxidative phosphorylation within cells. It is possible that the compounds of the invention preferentially target the same target as HIV and so resist or prevent the manifestations of infection including the procreative capacity of the virus.

20

Accordingly, the present invention in one aspect provides the use of a flavin, especially riboflavine, or a derivative thereof for the manufacture of a medicament for the management and treatment of viral infection.

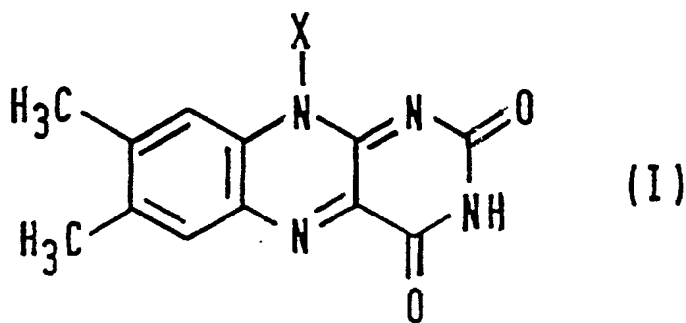
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Moreover, insofar as certain flavins and derivatives

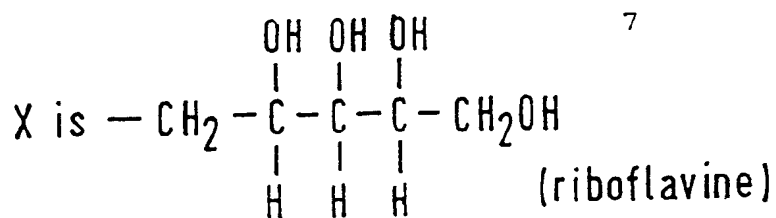
thereof are not known as pharmaceuticals, even in a general sense as with riboflavine (known as an enzyme co-factor vitamin), the invention in a second and broader aspect provides such certain flavins or a derivative thereof for use as anti-viral agents.

In the use according to the invention riboflavine or other flavin may be used as such or as a derivative and the flavin derivative may be any derivative which is safe for human or animal use. Preferably, however, in the case of riboflavine the derivative is a riboflavine salt and more preferably the riboflavine salt is riboflavine sodium phosphate or riboflavine tetrabutyrate. Most preferably, the flavin or derivative should be of high purity and contamination with spurious ingredients should be avoided.

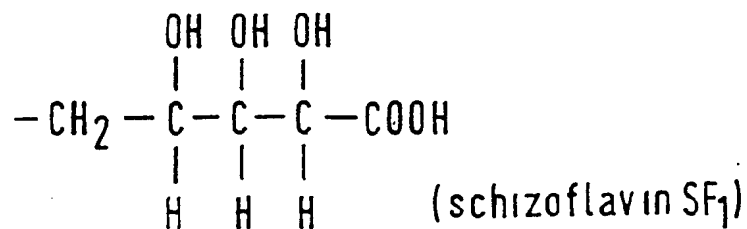
In more general terms, the flavin or derivative for use in accordance with the invention may be defined as a compound of the formula (I), namely:



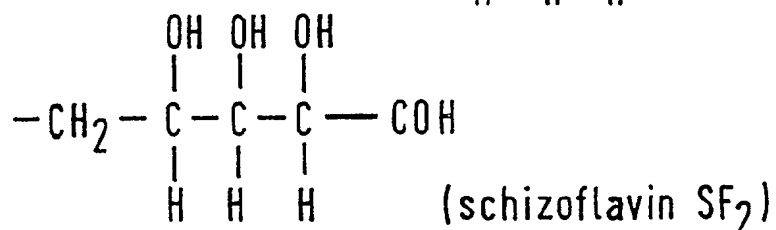
wherein:



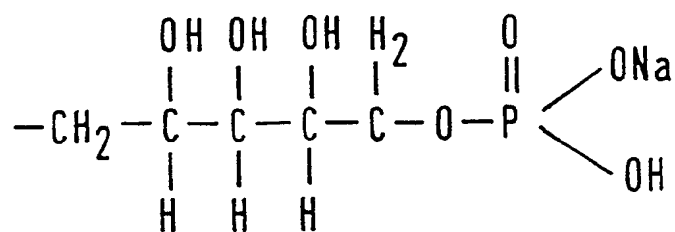
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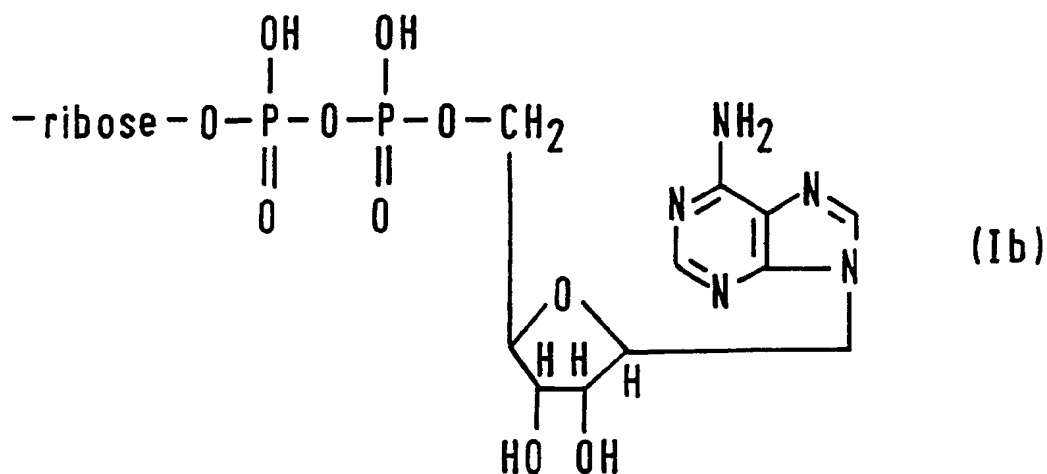


15



(riboflavine-5'-phosphate sodium salt dihydrate)

20



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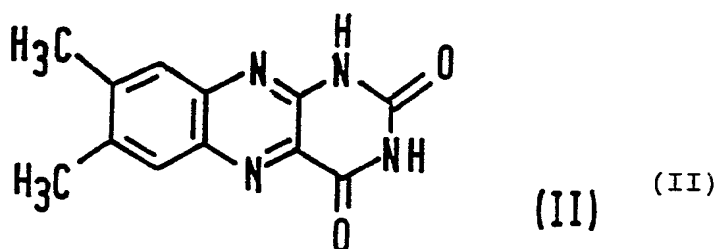
(flavin-adenine dinucleotide)

or  $\text{CH}_3$  (lumiflavin).

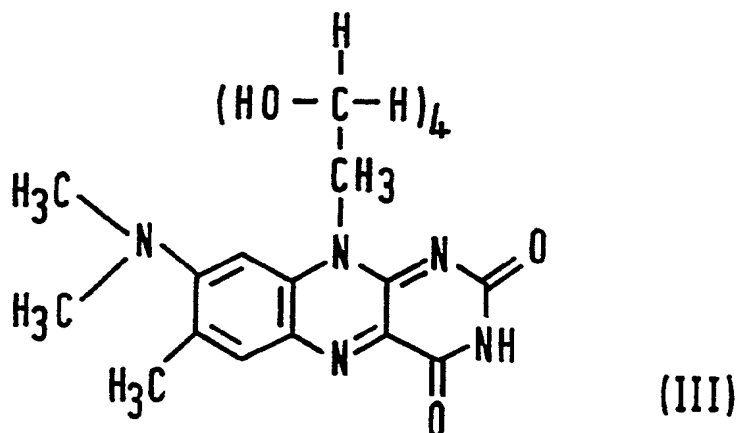
In addition, in the above formula (I) the group X may be alkyl, or H or an aromatic or other cyclic hydrocarbon group.

Thus, and furthermore, the use of the invention may be realised with flavins or derivatives such as:

(A) lumichrome of the formula:

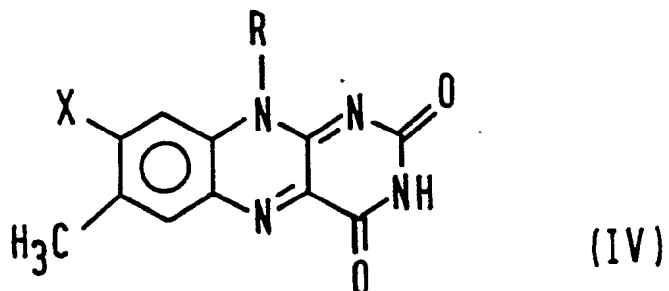


(B) Roseoflavin of the formula:



(C) B-Hydroxyflavine, alloxazines and other derivatives

thereof:

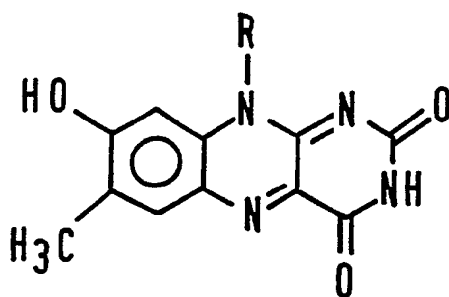


wherein

R is ribityl, alkyl, or H;

X is OH, Br, Cl, -SH, OAlk or SAlk.

Some Examples of the above are:

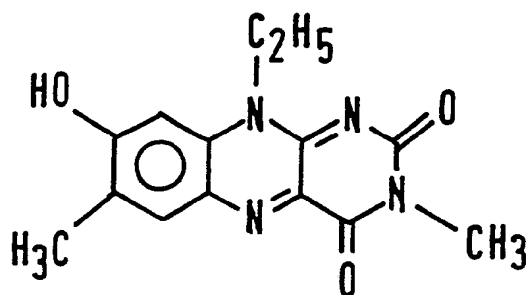


R = alkyl

ribityl

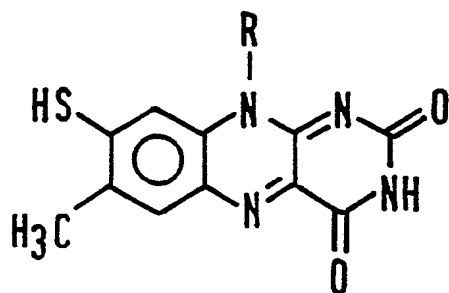
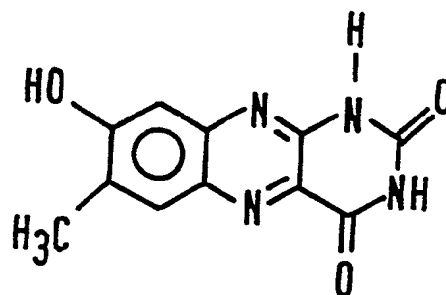
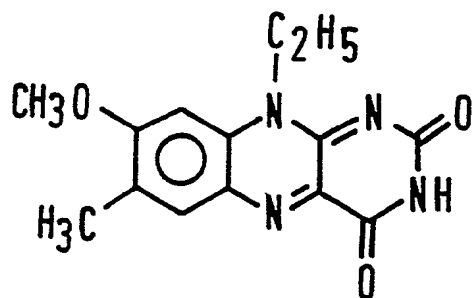
or rib-P

(8-hydroxy-FMN)



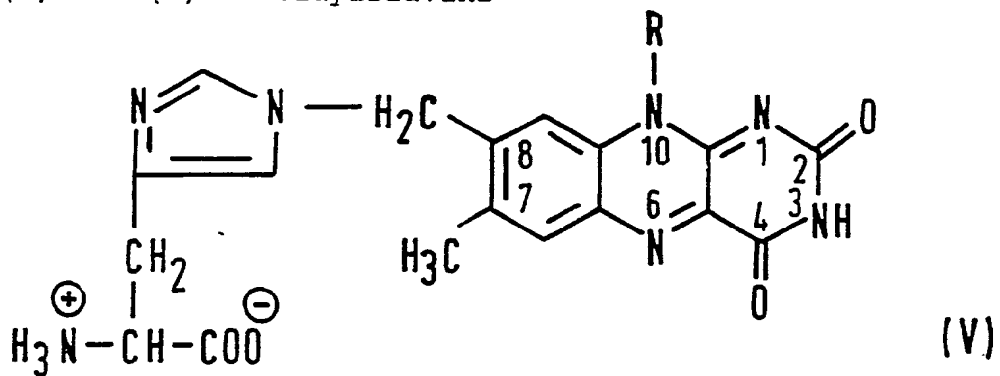
R=Rib-P-AMP

(8-hydroxy-FAD)



wherein R is as above.

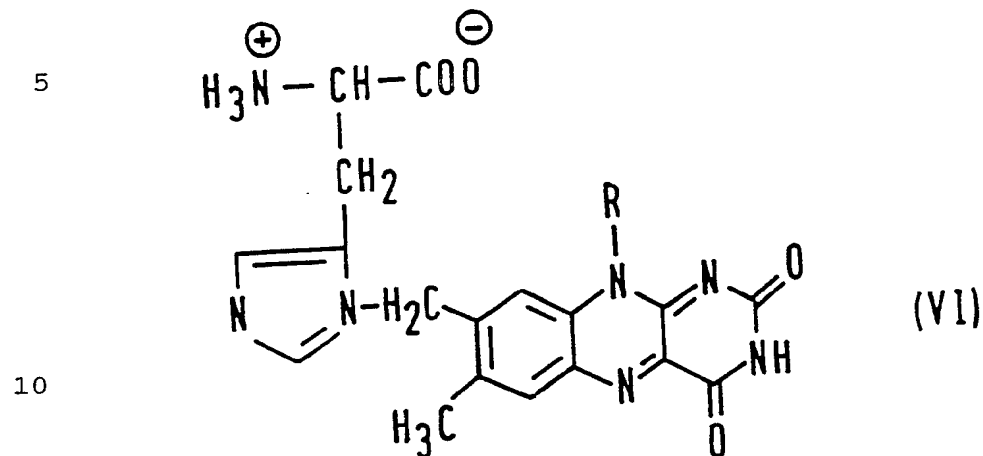
(D) 8 $\alpha$ -N(3)-histidylflavins



wherein R denotes the ribityl side chain for the riboflavin

derivative.

(E) 8 $\alpha$ N(1)-histidylflavins:

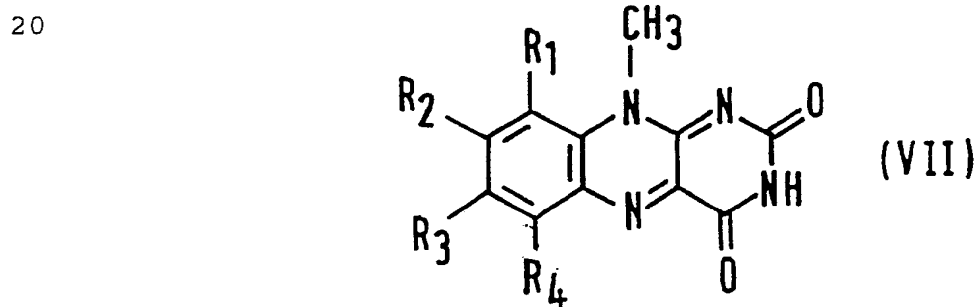


wherein R denotes the ribityl side chain for the riboflavin derivative.

15 (F) 8 $\alpha$ -Cysteinyflavin thioethers:

(G) 6-S-cysteinyflavin thioethers:

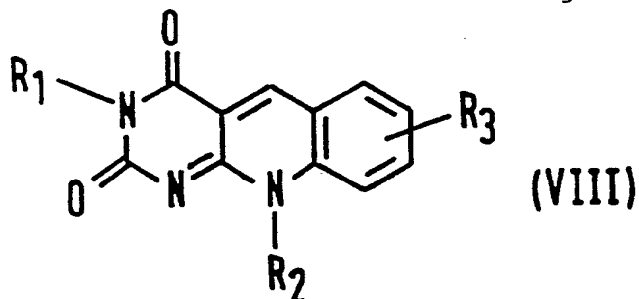
(H) Lumiflavins:



wherein  $R_1=R_4=H$ ,  $R_2=R_3=CH_3$  for lumiflavin itself.

(I) 5-Deazaflavins:

These may be illustrated by the following formula:



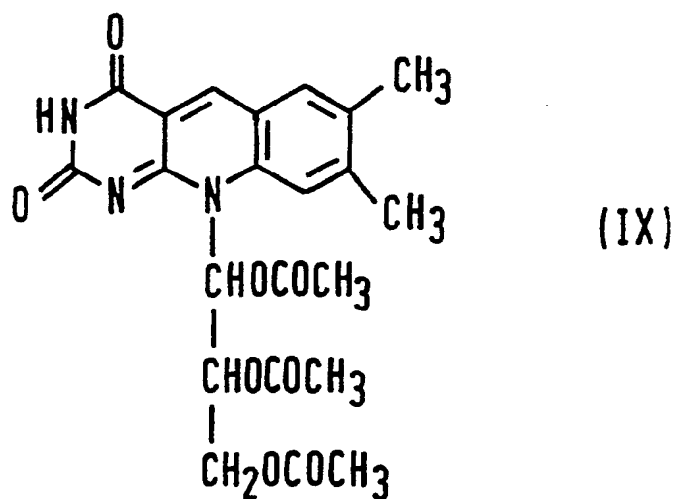
wherein the substituent groups are as defined below:

10

	$R^1$	$R^2$	$R^3$
	H	CH <sub>3</sub>	H
	H	C <sub>2</sub> H <sub>5</sub>	H
	H	n-C <sub>3</sub> H <sub>7</sub>	H
15	H	n-C <sub>4</sub> H <sub>9</sub>	H
	CH <sub>3</sub>	CH <sub>3</sub>	H
	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H
	CH <sub>3</sub>	n-C <sub>3</sub> H <sub>7</sub>	H
	CH <sub>3</sub>	n-C <sub>4</sub> H <sub>9</sub>	H
20	H	CH <sub>3</sub>	7,8-(CH <sub>3</sub> ) <sub>2</sub>
	H	D-ribityl	7,8-(CH <sub>3</sub> ) <sub>2</sub>
	H	C <sub>2</sub> H <sub>5</sub>	7-CH <sub>3</sub>
	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	7-CH <sub>3</sub>
	CH <sub>3</sub>	D-ribityl	7,8-(CH <sub>3</sub> ) <sub>3</sub>

25

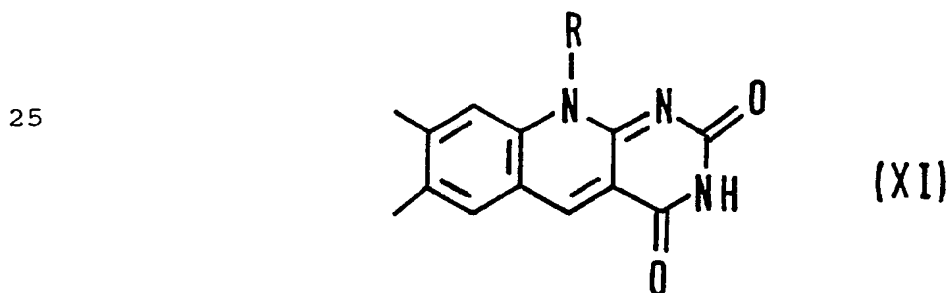
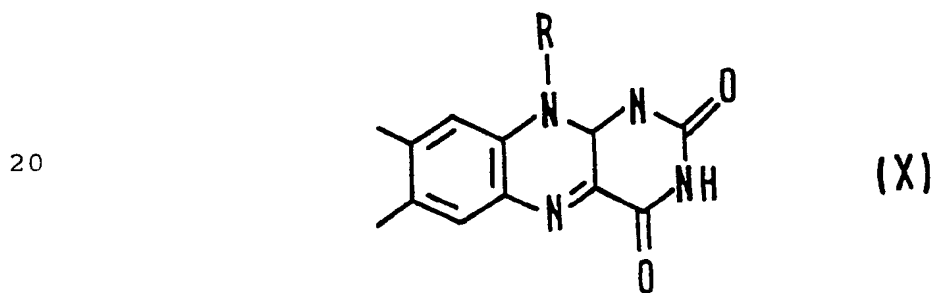
and derivatives thereof such as:

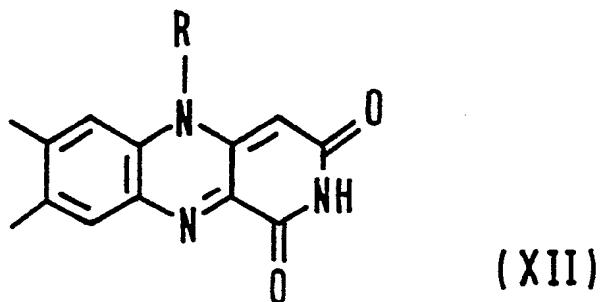


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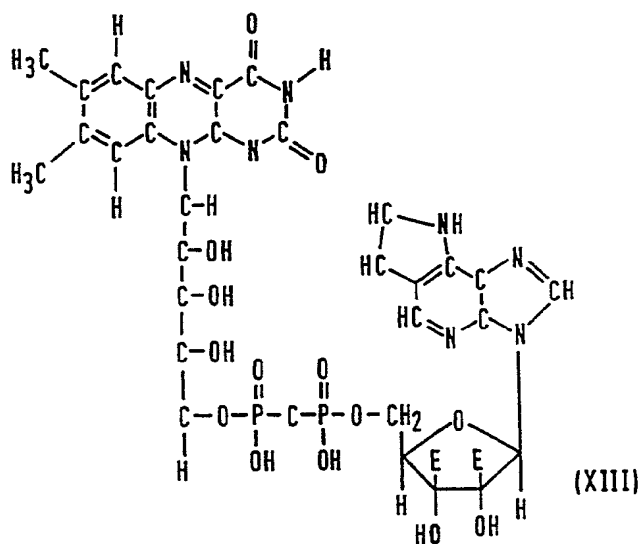
(J) 5-Carba-5-deaza and 1-carba-1-deaza analogs of riboflavin, FMN, and FAD.

These may be illustrated by riboflavin analogs (X), 5-carba-5-deazariboflavin analogs (XI) and 1-carba-1-deazariboflavin analogs (XII), that is:



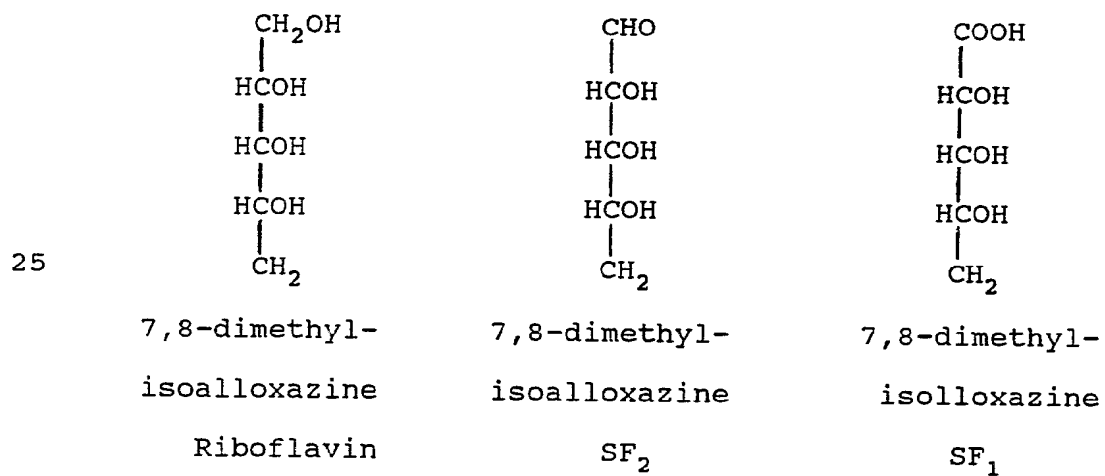


(K) Flavin 1, N<sup>6</sup>-Ethyoadenine dinucleotide



(L) Schizoflavins and derivatives.

20



The above are chemical structures of schizoflavins and show their formation from riboflavin. SF<sub>2</sub> and SF<sub>1</sub> can be identified as 7,8-dimethyl-10-(2,3,4-trihydroxy-4-formylbutyl) isoalloxazine and 7,8-dimethyl-10-(2,3,4-trihydroxy-4-carboxybutyl) isolloxazine, respectively.

Other flavins may be illustrated by:

3-carboxymethylriboflavin  
 3-carboxymethyl FMN  
 10 7-amino-10-(1'-D-ribityl) isoalloxazine  
 8-amino-7,10-dimethylisoalloxazine  
 8 $\alpha$ -(S-Mercaptopropionic acid) riboflavin  
 8 $\alpha$ -(S-Mercaptopropionic acid) FMN  
 8 $\alpha$ -(N-Aminohexyl) FMN  
 15 9-Azobenzoyl FMN  
 10-( $\omega$ -carboxyalkyl)-7,8-dimethylisoalloxazine

In the use according to the invention the flavin such as riboflavin, or derivative thereof, is preferably employed at a high dose level significantly in excess of the doses currently used or recommended. Thus, typically the riboflavin or other flavin in the clinical trial is used in the present invention at a dosage regime of at least about 1 to about 100 or more (eg 10 or above) mg/kg of body weight per day. In addition, use according to the invention preferably is one wherein the medicament is in orally administrable form, especially as a capsule (eg two-part).

Additionally or alternatively the invention includes a pharmaceutical or veterinary composition for use in the management and treatment of viral infections and in unit dosage form, which composition comprises a unit dose of at least about 35 mg such as 50mg or more (eg 50 to 300 mg, such as 50 to 200 or 50 to 100mg) of a flavin such as riboflavine or derivative thereof as described or defined herein, together with a pharmaceutically or veterinarily acceptable diluent, excipient or carrier.

10

A composition according to the invention is preferably one wherein the unit dose is from about 35 mg to about 1000 mg. More preferably, the unit dose is from about 250 to 500 mg.

15

In addition, a composition according to the invention is preferably in oral or injectable form. Within that context a preferred composition is one as a solution in sterile water.

20

The invention also includes a process for the manufacture of a medicament for use in the management and treatment of viral infections, which process comprises formulating a flavin such as riboflavine, or a derivative such as the tetrabutyrates salt thereof for anti-viral use.

25

As will be appreciated, a process according to the above definition may be carried out using one or more of the additional features mentioned herein.

In addition, the invention includes a product containing a flavin such as riboflavine, or a derivative thereof, as an anti-viral agent, together with another compound(s) having  
5 anti-viral activity as a combined preparation for simultaneous, separate or sequential use in anti-viral therapy.

Again, a product according to the above definition may be  
10 one which includes one or more of the other specific features of the invention defined herein.

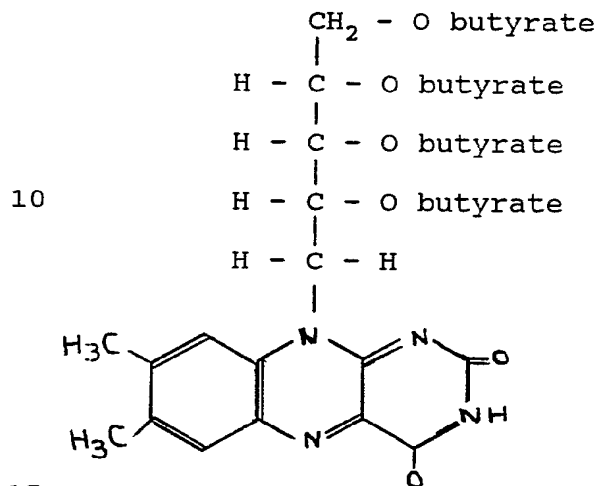
The invention further includes a method for the treatment of viral infection, which method comprises orally or  
15 parenterally administering an effective amount of a flavin such as riboflavine, or a derivation thereof.

Preferably in a method according to the invention, the amount administered is 1 to 100 (eg at least 10) mg/kg of  
20 patient body weight.

Furthermore, the method is particularly useful when the virus is human immunodeficiency virus, HIV.

25 Once again, a method according to the invention may include one or more of the other specific features of the invention defined herein.

Most preferably, the invention is carried out with one or more of riboflavine, riboflavine sodium phosphate, flavin-adenine dinucleotide, lumiflavin, lumichrome, or especially riboflavin tetrabutrate, whose formula is set forth below:-



### In Vitro Assay

20 The following in vitro assays were used to investigate the anti-viral activity against HIV of compounds in accordance with the invention:-

#### 1 Acute Infection Assays

##### 25 1.1 Standard Acute Assay

High titre virus stocks of the human immunodeficiency virus HIV-1 (HTLV-111B) were grown in H9 cells with

RPMI 1640 supplemented 10% fetal calf serum as growth medium. Cell debris was removed by low speed centrifugation and the supernatant stored at  $-70^{\circ}\text{C}$  until required. In a typical assay, C8166 T-lymphoblastoid cells were incubated with  $10\text{TCID}_{50}$  HIV-1 at  $37^{\circ}\text{C}$  for 90 minutes and then washed three times with phosphate buffer saline (PBS). Aliquots of  $2 \times 10^5$  cells were resuspended in 1.5ml of growth medium in 6ml culture tubes, and test compound at log dilutions from 0.2 to  $200\mu\text{M}$  was added immediately. The test compound was dissolved in 70% ethanol and the final concentration of alcohol in the culture was  $<1\%$ . Cultures were incubated at  $37^{\circ}\text{C}$  for 72 hours in  $5\% \text{CO}_2$ .  $200\mu\text{l}$  of supernatant was taken from each culture and assayed by optical density measurement at 450nm for HIV p24 core antigen (Kinchington et al 1989, Roberts et al 1990) using a commercial ELISA which recognises all the core proteins equally (Coulter Electronics Ltd, Luton, UK). To determine the  $\text{IC}_{50}$  values standard curves were drawn from untreated cultures containing  $<1\%$  alcohol. AZT and ddC were used as internal controls. Assays were carried out in duplicate.

### 1.2 Depleted Medium Assay

In the standard assay system, cell cultures were harvested, split and fed with fresh medium approximately 18 to 24 hours before the start of

assay. Addition of fresh medium stimulates the cells to enter a log phase of growth. To investigate the effect of cells reaching confluence in conditions of depleted media, cell cultures were fed and split at 72, 48 and 24 hours before being used in a standard acute assay.

### 1.3 Light Exposure Assay

A freshly dissolved sample of test compound was split into two aliquots. They were placed either in daylight or the dark for two hours before being subjected to standard acute assay.

### 1.4 Preincubation Assay

Target cells were preincubated with test compound at log dilutions of 200 to 0.2 $\mu$ M for 18/24 hours before infection with HIV-1. Each sample concentration was then treated individually as in the standard acute assay.

## 2 Assays for Chronically Infected Cells

### 2.1 Standard Chronic Assay

H9 cells chronically infected with HIV-1rf (H9rf) were washed three times with medium to remove extracellular

virus and incubated with test compounds (200 to 0.2 $\mu$ M) for three days. p24 antigen was then determined by optical density measurement at 450nm as described for the acute infection standard assay. To determine the IC<sub>50</sub> values standard curves were drawn from untreated cultures containing 1% alcohol. RO 31-8959 (Roche Proteinase inhibitor) was used as an internal control. Assays were carried out in duplicate.

## 10 2.2 Depleted Medium Assay

In the standard assay, cell cultures were harvested, split and fed with fresh medium approximately 18 to 24 hours before assay. Addition of fresh medium stimulates the cells to enter a log phase of growth. To investigate the effect of cells reaching confluence in conditions of depleted media, cell cultures were fed and split at 72, 48 and 24 hours before being used in a standard acute assay.

20

## 2.3 Light Exposure Assay

A freshly dissolved sample of test compound was split into two aliquots. They were placed either in daylight or the dark for two hours before being subjected to standard chronic assay.

### 3      Toxicity Assay

To test for compound toxicity, aliquots of  $2 \times 10^5$  uninfected cells were cultured with test compounds at the same log dilutions for 72 hours (1.1 and 2.1). The cells were then washed with medium and resuspended in 200 $\mu$ l of growth medium containing  $C^{14}$  protein hydrolysate. The cells were harvested after 5 or 20 hours and the  $C^{14}$  incorporation measured. Untreated cells were used as controls.

The assays were applied to the compounds identified in Table 1 below:-

Table 1

<u>Code</u>	<u>Compound</u>
F1	Riboflavine
	5'phosphate
F2	Riboflavine
F3	Flavine adenine
	dinucleotide
F4	Lumiflavin
F5	Lumichrome
F6	Riboflavin tetranicotinate
F7	Riboflavin tetrabutyrate

Initial assays were carried out in relation to the various compounds mentioned in Table 2 to achieve preliminary

results. The  $IC_{50}$  results in Table 2 are subject to confirmation; they are not consistent with re-run assays conducted to date. Assay results are shown in the graphs forming the following drawings and in Tables 2 to 10 which follow:-

Figure 1: Antigen as optical density (OD) for Compounds F2, F4 (first antigen assay) and F5 at 450 nm versus concentration ( $\mu M$ ). The dotted line at OD 0.371 represents  $IC_{50}$  (active).

Figure 2: Antigen optical density (OD) for Compounds F1 and F3 at 450 nm versus concentration ( $\mu M$ ). The dotted line at OD 0.371 represents  $IC_{50}$  (active).

Figure 3: Toxicity as  $C^{14}$  uptake (dpm) versus concentration ( $\mu M$ ) for Compounds F2, F3, F4 (first toxicity assay) and F5. The dotted line at 6035 dpm represents  $CC_{50}$  (non-toxic).

Figure 4: Toxicity as  $C^{14}$  uptake (dpm) versus concentration ( $\mu M$ ) for Compound F1. The dotted line at 6035 dpm represents  $CC_{50}$  (non-toxic).

Figure 5: Antigen optical density (OD) for Compound F4 (second antigen assay) at 450 nm versus concentration ( $\mu M$ ). The dotted line at OD 0.371 represents  $IC_{50}$  (active).

Figure 6: Toxicity as  $C^{14}$  uptake (dpm) versus concentration ( $\mu M$ ) for Compound F4 (second toxicity assay). The dotted line at 6035 dpm represents  $CC_{50}$  (non-toxic).

Figure 7: Antigen optical density (OD) for Compounds F6 and F7 at 450 nm versus concentration ( $\mu M$ ). The dotted line at OD 0.371 represents  $IC_{50}$  (active).

Figure 8: Toxicity as  $C^{14}$  uptake (dpm) versus concentration ( $\mu M$ ) for Compounds F6 and F7. The dotted line at 6035 dpm represents  $CC_{50}$  (non-toxic).

Figure 9: Antigen control (ddC)

As shown by the Tables, the test compounds were evaluated for activity against cells both acutely and chronically infected with HIV. Antiviral ( $IC_{50}$ ) and toxicity ( $CC_{50}$ ) data is shown below. In another series of experiments, compounds were tested in cell cultures in which fresh media was added at 72, 48 and 24 hours prior to infection. This experiment was carried out to investigate the effects of the compounds on cells in actively dividing and quiescent states. This data indicates that cells may be more sensitive to the test compounds when quiescent. The effect of light on stability, preincubation of target cells, and the activity against an African HIV-1 isolate were also

investigated. Exposure to light for two hours had no effect on the activity of the compound. Preincubation with the target cells enhanced its activity and it showed significant activity against the Africa HIV-1 isolate.

5

*Table 2 (Figures 1 to 4) - Acute Infection Standard Assay (1.1)*

10	<u>Compound No/ Assay No</u>	<u>IC<sub>50</sub></u> (Figures 1 and 2)	<u>CC<sub>50</sub></u> (Figures 3 and 4)	<u>SI</u>
	F1/1	1 to 20	>200	-
	F1/2	<0.4	>400	>1000
	F1/3	0.1 (Figure 2)	>800 (Figure 4)	>8000
15	F2	3 (Figure 1)	>200 (Figure 3)	>60
	F3	0.8 (Figure 2)	>200 (Figure 3)	>200
	F4	1 (Figure 1)	150 (Figure 3)	150
	F5	3 (Figure 1)	>200 (Figure 3)	>60

20 *Table 3 (Figures 7 and 8) - Acute Infection Standard Assay (1.1)*

25	<u>Compound No/ Assay No</u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>SI</u>
	F7/1	27 (Figure 7)	130 (Figure 8)	5
	F7/2	57	>200	>4
	F7/3	10	70	7
	F7/4	25	140	6

30

Table 4 - Chronic Infection Standard Assay (2.1)

5	<u>Compound No/ Assay No</u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>SI</u>
	F7/1	0.2	7	35
	F7/2	>20	>20	-
	F7/3	10	>200	>20
	F7/4	4	75	19
10	F7/5	26	>200	>7

Table 5 - Acute Infection Depleted Medium Assay (1.2)

15	<u>Compound No</u>	<u>72 hours</u>		<u>48 hours</u>		<u>24 hours</u>	
		<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>
	F7	10	160	21	100	110	160

20 Table 6 - Chronic Infection Depleted Medium Assay (2.2)

25	<u>Compound No</u>	<u>72 hours</u>		<u>48 hours</u>		<u>24 hours</u>	
		<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>
	F7	40	75	90	250	60	101

Table 7 - Acute Infection Light Radiation Exposure Assay (1.3)

30

	<u>Compound No</u>	<u>Daylight</u>		<u>Darkness</u>	
		<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>
	F7	60	>200	60	>200

35

Table 8 - Acute Infection Preincubation Assay (1.4)

Preincubation of target cells with test compound for 24 hours before infection

<u>Compound No</u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>SI</u>
F7	5	120	24

Table 9 (Figures 5 to 8) - Acute Infection Standard Assay (1.1)

<u>Compound No</u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>SI</u>
F4	13 (Figure 5)	150 (Figure 6)	12
F6	30 - 60 (Figure 7)	>200 (Figure 8)	min 3 -6

Table 10 - Acute Infection Standard Assay (1.1)

Assay applied to C8166 Cells (T-lymphoblastoid cells transformed and immortalized by HTLV) with an African HIV Isolate (HIV-1 CBL4)

<u>Compound No</u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>SI</u>
F7	4	150	37.5

Table 11 (Figures 10 to 12) - Acute Infection Standard Assay (1.1)

<u>Compound No</u>	<u>IC<sub>50</sub></u>	<u>CC<sub>50</sub></u>	<u>SI</u>
F7	32	200	6.3
ddC (control)	0.2		

The variation in the end points observed with Compound F7 may be due to the properties of the target lymphoblastoid

cells. Even in synchronized cultures there may be subtle changes in the metabolic state of sub-populations of cells. This is reflected in the shift in the end points observed in the paired antiviral and toxicity values from assay to assay (Table 3). The results tabulated in Tables 5 and 6 indicate that cell culture in active or quiescent states may have different sensitivities to the test compound.

### Patient Treatment

Thirty-five patients were placed on therapy. Thirty had follow up medical visits.

#### i) General Condition of the Patients

Twenty patients out of thirty who came for follow-up visits reported an improvement in their general condition. The majority of these reported improvement insofar as malaise, appetite and weight gain was concerned. Two patients also reported improvement in skin rash with regression of skin lesions while one reported no new skin lesions developed while on therapy. One patient also reported improvement in impotence (which had been present for three months prior to onset of therapy), while two other patients reported cessation of long standing coryza.

ii) Sick Visits

Few patients attended clinic for unscheduled sick visits:-

- 5      1.    One patient had recurrent abscesses as well as septic arthritis which persisted even on therapy.
- 10      2.    Two patients had recurrent lower respiratory tract infections with one developing frank broncho-pneumonia during second week of therapy. Repeated smears for AAFBS have continued to be negative.
- 15      3.    Two patients had pyrexia with no localizing signs and repeated blood smear for malarial parasites were negative and no significant growth on blood culture. One of these patients responded well to amoxycillin and is now afebrile.
- 20      4.    One patient had gastroenteritis during the third week of therapy.
- 25      5.    Oral and vulvo-vaginal candidiasis were reported by two patients, with the vulvo-vaginal candidiasis being recurrent as soon as a course of Nystatin pessaries and tablets was completed.
6.    Two patients also reported recurrent attacks of herpes simplex genitalis.

iii) Toxicity

Most of the cases of toxicity reported occurred during the first two weeks of therapy and have been transient.

5

Two patients experienced pruritus which averaged four days during first week of therapy and cleared spontaneously without any supportive medication.

10 Four patients reported moderate diarrhoea during the first two weeks of therapy. This has averaged four days. This has been a difficult symptom to attribute as between it being a side effect or a natural manifestation of the HIV infection. However, the consistency of its appearance in  
15 the first week of therapy, and its transient nature makes it reasonable to suppose it is a side effect.

One patient reported drowsiness and another reported darkening of her urine. MSU was normal.

20

Two patients reported abdominal discomfort.

iv) Laboratory Results

25 Three patients had transient rises in liver enzymes during the second to third week of therapy, with no clinical signs of liver disease. However, the enzyme levels always returned to normal.

The above clinic trial reports are the preliminary results of a clinical trial which has currently been in progress for several weeks using Compound F7 administered orally in capsule form (the capsules are as described in Example 4 below) dosage was:-

Dose level 1: 1mg/kg body weight per day orally in two divided dosages

10 Dose level 2: 2mg/kg body weight per day orally in two divided dosages

Dose level 3: 10mg/kg body weight per day orally in two divided dosages

15 Dose level 4: 15mg/kg body weight per day orally in two divided dosages

Dose level 5: 20mg/kg body weight per day orally in two divided dosages

Dose level 6: 30mg/kg body weight per day orally in two to three divided dosages

20 Dose level 7: 40mg/kg body weight per day orally in two to three divided dosages

Dose level 8: 50mg/kg body weight per day orally in two to three divided dosages

25 Dose level 9: 100mg/kg body weight per day orally in two to three divided dosages

The following specific Examples illustrate compositions formulated in accordance with the invention:-

Example 1

A formulation can be prepared from the following:

5	riboflavine-5-phosphate	10 mg
	sterile water	2 ml

to provide a unit dosage of 10 mg of riboflavine for  
administration once per day in the treatment of viral  
10 infection.

Example 2

A formulation can be prepared from the following:

15	riboflavine-5-phosphate	30 mg
	sterile water	2 ml

to provide a unit dosage of 30 mg of riboflavine for  
administration once per day in the treatment of viral  
20 infection.

Example 3

25 Similar formulations to those of Examples 1 and 2 can be  
prepared at doses of:

10 mg per ml,

25 mg per ml, and

50 mg per ml

respectively, in either a unit amount of 2 ml or 5 ml of  
5 sterile water and based on an active ingredient which is:

Riboflavine 5'phosphate

Riboflavine

Flavine adenine dinucleotide

10 Lumiflavin

Lumichrome

or a mixture thereof.

15 Example 4

The following capsules were formulated:-

Sizes: 25mg  
20 50mg  
100mg  
200mg  
400mg

25 Type: 2-part hard gelatin

Composition: Compound F7 in admixture with  
microcrystalline cellulose Ph. Eur

166.4/156.7/118.6/108.7/50mg to give capsule  
weights of 191.4/206.7/218.6/ 308.7/450mg.

CLAIMS

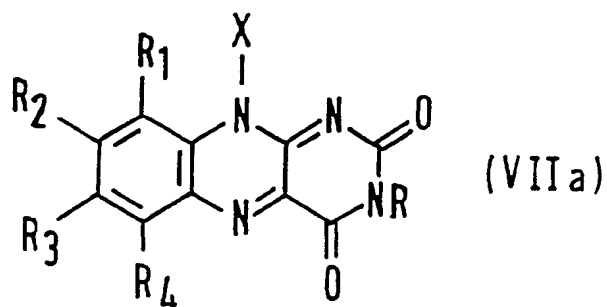
1. Use of a flavin, flavin derivative or a mixture comprising two or more thereof for the manufacture of a medicament for the treatment by prophylaxis or therapy of disease caused by viral infection.

2. Use as claimed in Claim 1 wherein the flavin derivative is riboflavin or a riboflavin derivative.

3. Use as claimed in Claim 2 wherein the riboflavin derivative is a riboflavin salt.

4. Use as claimed in Claim 3 wherein the riboflavin salt is riboflavin sodium phosphate or riboflavin tetrabutyrates.

5. Use as claimed in Claim 1 wherein the flavin or flavin derivative is a compound of the general formula:-

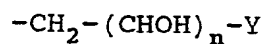


wherein R is hydrogen or alkyl;

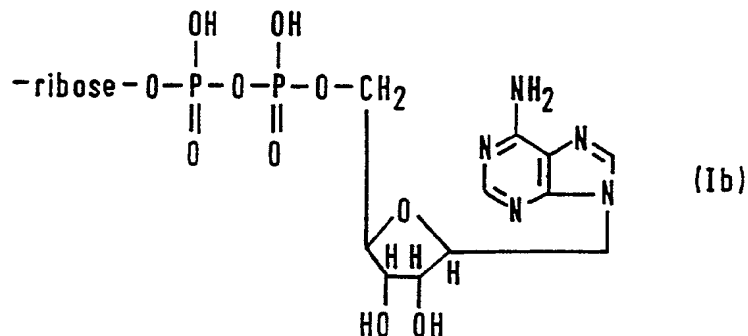
R<sub>1</sub> and R<sub>4</sub> are, each independently, hydrogen, alkyl, hydroxy, halo, alkoxy, alkylthio, thio or an optionally substituted aromatic or non-aromatic nitrogen heterocycle, and X is:-

(i) hydrogen, ribityl, alkyl, hydrogen or an aromatic or non-aromatic carbocycle

5 (ii) a group of the general formula:-



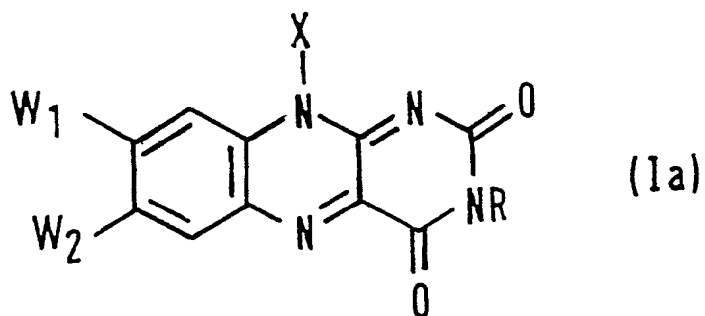
in which n is an integer of 3 or 4 and Y is  $-\text{CH}_2\text{OH}_1-\text{COOH}$  or  $-\text{COH}$  or a group of the formula:-



wherein R is hydrogen or alkyl; and wherein  $W_1$  and  $W_2$  are, each independently, alkyl, hydroxy, halo, alkoxy, alkylthio, thio or an optionally substituted aromatic or non-aromatic nitrogen heterocycle.

20

6. Use as claimed in Claim 1 wherein the flavin or flavin derivative is a compound of the general formula:-



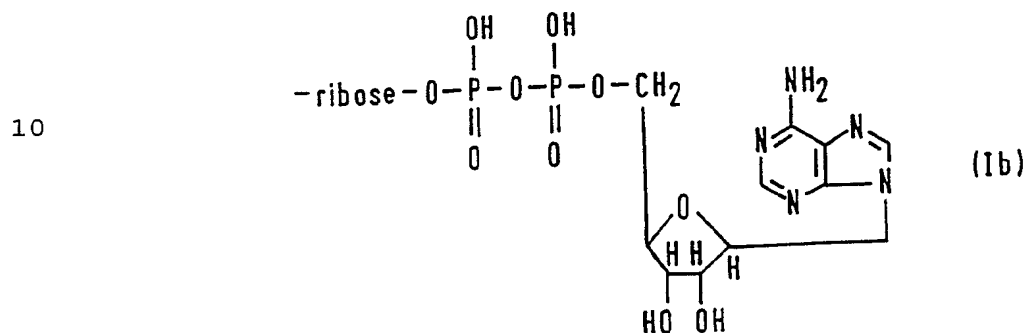
wherein X is

(i) hydrogen, ribityl, alkyl, hydrogen or an aromatic or non-aromatic carbocycle

(ii) a group of the general formula:-

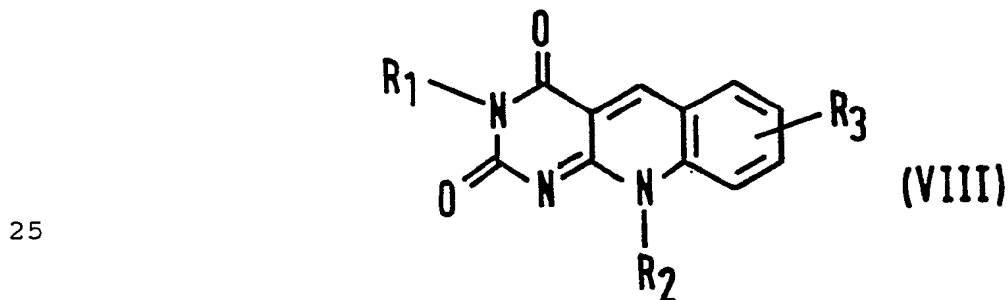


in which n is an integer of 3 or 4 and Y is  $-\text{CH}_2\text{OH}_1-\text{COOH}$  or  $-\text{COH}$  or a group of the formula:-



15 wherein R is hydrogen or alkyl; and wherein  $W_1$  and  $W_2$  are, each independently, alkyl, hydroxy, halo, alkoxy, alkylthio, thio or an optionally substituted aromatic or non-aromatic nitrogen heterocycle.

20 7. Use as claimed in Claim 1 wherein the flavin or flavin derivative is a compound of the general formula:-



wherein  $R_1$  is hydrogen or an alkyl group,

$R_2$  is an alkyl group or a ribityl group, and  
 $R_3$  represents hydrogen or mono- or di-substitution of the  
outer carbocyclic ring with an alkyl group.

5        8. Use as claimed in Claim 1, wherein the flavin or  
flavin derivative is lumichrome; roseflavin; a  
hydroxyflavin; an alloxazine or derivative thereof; an  $8\alpha$ -  
N(3)-histidylflavin; an  $8\alpha$ -N(1)-histidyl flavin; an  $8\alpha$ -  
cysteiny1 thioether; an  $6\alpha$ -S-cysteiny1 thioether; a  
10       lumiflavin; a 5-deazaflavin; a 5-carba-5-deaza or 1-carba-  
1-deaza analog of riboflavin, FMN or FAD; flavin-1,N<sup>6</sup>-  
ethenoadenine dinucleotide; 9-methylflavin; 9-phenylflavin;  
9-benzylflavin; 9-cyclohexylflavin; 6,9-dimethylflavin;  
6,7,9-trimethylflavin; 9-oxyethylflavin; 9-  
15       dioxypropylflavin; 6,8,9-trimethylflavin; lacroflavin;  
flavin-9-carboxylic acid; 6,7-dimethylflavin-9-carboxylic  
acid; or a schizaflavin.

20       9. Use as claimed in any preceding claim at a dosage  
regime of at least about 10 mg/kg of body weight per day.

10. Use as claimed in any preceding claim wherein the  
medicament is in injectable form.

25       11. A flavin or flavin derivative for use in the  
manufacture of a medicament useful in the treatment by  
prophylaxis or therapy of disease caused by viral  
infection.

12. A flavin or flavin derivative as claimed in Claim 11 and as defined in any one of Claims 2 to 8.

5 13. A pharmaceutical composition for the treatment by prophylaxis or therapy of disease caused by viral infection, the composition being characterized in that it comprises a flavin or flavin derivative.

10 14. A composition as claimed in Claim 13 wherein the flavin or flavin derivative is as defined in any one of Claims 2 to 8.

15 15. A composition as claimed in Claim 12 or Claim 13 which composition comprises a unit dose of at least about 35 mg of a flavin or flavin derivative together with a pharmaceutically or veterinarily acceptable diluent, excipient or carrier.

20 16. A composition as claimed in Claim 15 wherein the unit dose is from about 35 mg to about 1000 mg.

17. A composition as claimed in Claim 16 wherein the unit dose is from about 250 to 500 mg.

25

18. A composition as claimed in any one of Claims 15 to 17 which is in injectable form.

19. A composition as claimed in Claim 18 in the form of a solution in sterile water.

5 20. A receptacle for pharmaceutical containment immediately pre-administration, said receptacle being manipulable in a drug administration procedure by medical practitioners and containing a flavin or flavin derivative for discharge from the receptacle to a patient or to an administration device and said receptacle carrying a  
10 representation of instructions for use of the flavin or flavin derivative as a medicament for the treatment by prophylaxis or therapy of disease caused by viral infection.

15 21. The combination of :-

(a) a flavin or flavin derivative formulated for pharmaceutical use, and

20 (b) instructions for use of said formulated flavin or flavin derivative for the manufacture of a medicament for the treatment by therapy or prophylaxis of disease caused by viral infection or for use thereof for said treatment.

25 22. The combination of Claim 21 wherein the treatment is referred to in the instructions and is the treatment of HIV-infection.

23. The combination of Claim 22 wherein the HIV-infection is chronic infection.

24. A process for the manufacture of a medicament for use  
5 in the management and treatment of viral infection, which process comprises formulating a flavin or flavin derivative for anti-viral use.

25. Flavin, or a flavin derivative as an anti-viral agent,  
10 together with another compound(s) having anti-viral activity, as a combined preparation for simultaneous, separate or sequential use in anti-viral therapy.

26. A method for the treatment by prophylaxis or therapy  
15 of disease caused by viral infection which method comprises administering therapeutically to a patient suffering from such disease an effective amount of a flavin or a flavin derivative or administering prophylactically to a patient at risk of viral infection an effective amount thereof.

20

27. A method as claimed in Claim 26 wherein the amount administered is at least about 1 to about 10 or more mg/kg of patient body weight.

28. A flavin or a flavin derivative thereof not known for  
25 any pharmaceutical utility for use as an anti-viral agent.

29. A flavin or flavin derivative for use in the treatment

by prophylaxis or therapy of a disease caused by viral infection.

5 30. A flavin or flavin derivative as claimed in Claim 9 and as defined in any one of Claims 1 to 8.

10 31. An anti-viral agent for use in the treatment of HIV-infection in a mammalian subject at least at a chronic infection stage, the agent having a cellular target and optionally also a viral target and being a flavin or flavin derivative acting intracellularly on cell metabolism in mammalian cells infected chronically or acutely with HIV to block or compensate for the effects of the viral infection on the cell in the asymptomatic and post-asymptomatic phases of the infection by the virus.

15

32. An anti-viral agent as claimed in Claim 31 and which is a riboflavin derivative.

20 33. A method of in vitro diagnostic assay which method comprises sampling the cells of a mammalian patient infected with HIV after treating the patient by a treatment regime in which a flavine or flavine derivative is administered to the patient, and performing an assay upon the cell sample externally of and separate from the patient's body to determine the activity and/or progress of the viral infection.

25

**Patents Act 1977**  
**Examiner's report to the Comptroller under Section 17**  
**(The Search report)**

43

Application number  
GB 9421099.4

**Relevant Technical Fields**

- (i) UK Cl (Ed.N)      A5B (BHA, BJA)  
(ii) Int Cl (Ed.6)      A61K 31/525

Search Examiner  
J F JENKINS

Date of completion of Search  
3 FEBRUARY 1995

**Databases (see below)**

(i) UK Patent Office collections of GB, EP, WO and US patent specifications.

(ii) ONLINE DATABASES: WPI, DIALINDEX (MEDICINE), CAS-ONLINE

Documents considered relevant following a search in respect of Claims :-  
1 TO 25, 28, 29, 31 TO 33

**Categories of documents**

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Category	Identity of document and relevant passages	Relevant to claim(s)
X	WO 93/10784 A1 (UNIV. MICHIGAN) see page 6 lines 8 to 23, page 8 lines 12 to 31, Claims 2 to 5 and 13	13 to 19, 28 to 32
X	WO 93/05784 A1 (EISAI) see WPI Abstract No. 93-117230/14	13 to 19, 28 to 32
X	WO 92/17173 A2 (BERQUE) whole document	1 to 4, 9 to 25, 28 to 33
X	US 4500524 (CATSIMPOOLAS) see Claims 1 and 2	13 to 19, 28 to 32
X	US 4264601 (TRACHEWSKY) see Claims 1 to 8	13 to 19, 28 to 32
X	US 4219545 (COLLINS) see Claims 1 to 5	13 to 19, 28 to 32
X	BIOSIS No. 96081026 (ABRAMS ET AL)	1, 2, 11 to 15, 20 to 24, 28 to 33
X	Martindale, The Extra Pharmacopolia, 29th Edn (1989) page 1272 "Uses"	13 to 19, 28 to 32

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